Introduction
Natural killer (NK) cells are integral to the innate immune system and function to immediately recognize and lyse virally infected cells and tumor cells. NK cell therapy is largely and solely directed towards liquid tumors rather than solid tumors. Solid tumors, including glioblastoma and pancreatic ductal adenocarcinoma, exhibit severe tumor hypoxia and maintain a suppressive tumor microenvironment through the secretion of NK sensitive-inhibitory cytokines.

TGF-β is an immunosuppressive cytokine released by tumor cells into the microenvironment and generally functions as an inhibitory response to innate immune response of NK cells. Oncolytic viruses are genetically-engineered viruses to specifically target and infect tumor cells and rather than normal cells. This includes ‘Oncolytic-Herpes Simplex Virus (oHSV)’ and ‘Δ24RGD (Adenovirus).’

Methods

Viral Pretreatment of Tumor Cells

Infected Tumor Preexposure Assay

TGF-beta - NK Cell Pretreatment and Titer Assay

Experimental Questions and Results:
1. Can oncolytic viruses stimulate NK cell killing of solid tumors?
2. How do oncolytic viruses enhance NK cell killing?
3. Why does the TGF-beta knockout + virally pretreated tumor cells exhibit the greatest NK cell killing response?

Figure 1: NK+Virus increases the cytotoxicity of NK cells against pancreatic tumor cells (PDAC).
Day 3 Incucyte fluorescence imaging of BXPC3. 1:10 effector to target ratio. Green fluorescence highlights live tumor cells and red, the dead cell stain. delta24-RGD, 0.5 MOI.

Figure 2: Combination of NK and delta24RGD demonstrates synergistic killing of glioblastoma stem cells (GSC8-11).
XCelligence of PATC148. oHSV tumor pretreatment, 0.05 MOI. 1:2 effector to target ratio.

Figure 3: Combination of NK and oHSV demonstrates synergistic killing of pancreatic tumor cells (PATC148).
XCelligence of PATC148. oHSV tumor pretreatment, 0.01 MOI. 2:1 effector to target ratio.

Conclusions
- Oncolytic viruses enhance the NK cell killing response of solid tumors.
- Dual Knockout NK Cells + Virally pretreated tumors exhibit the greatest NK cell tumor lysis.
- NK cells exhibit memory-like behavior upon infected tumor preexposure.

Future Directions
- Optimization of the TGF-beta pretreatment assay to better understand the mechanism behind the dual knockout enhanced NK cell tumor lysis.
- ATAQ Seq and RNA Seq to understand changes in gene expression at different timepoints within our preexposure assay.

References

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